(x ± SEM; n = 15 experiments). This was significantly ($p < 0.05$) different from the control value, which averaged 2.8 ± 0.3 days (n = 15). Additional analysis of human atherectomy specimens, from 32 coronary and 32 femoral plaques of primary (45) and restenotic (19) origin, showed SMC outgrowth in 43/64 cases (67%). Interestingly, previous AI therapy was followed by significantly ($p < 0.05$) lower success, whereas medication with β-blockers, Ca-antagonists, nitrates, aspirin or antilipids had no effect. Complementary experiments using isolated human and rat SMCs revealed no inhibition for benazeprilat (24 h preincubation) on growth curves and chemokinesis of these cells.

We conclude that long-term ACE inhibition strongly induces apoptosis of vascular SMCs which apparently explains the modified SMC outgrowth activity. Our data suggest ACE inhibitors to be attractive candidates for the regulation of growth processes *via* apoptosis, though previous clinical trials (without pretreatment period) have as yet failed to prevent restenosis.

1043-100 Ion Channel Modulators Differentially Regulate Proliferation and Apoptosis of Human Plaque Smooth Muscle Cells

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Proliferation of smooth muscle cells (SMCs) is considered to be a central mechanism involved in human arteriosclerosis, which is possibly modulated by transmembrane ion fluxes. Therefore, using SMCs cultivated from human plaque tissue removed by directional atherectomy, we screened different ion channel modulators for their potential, antagonistic effects on SMC proliferation. Growth curves were based upon directly measured cell signals (CASY 1), to quantitate population doubling rates (PDRs). For the testing of ion channel modulators, the PDRs of treated SMCs were compared to those of control cells (= 100%):

Ion Channel Modulator	Agent	Conc.	PDR
Ca^{2+} channel agonist	Bay K 8644	10^{-8} M	174%*
		10^{-7} M	205%**
Ca^{2+} channel antagonist	nicardipine	10^{-8} M	85% ns
		10^{-7} M	78%*
		10^{-5} M	47%**
K^+ channel activator	nicorandil	10^{-8} M	77%*
		10^{-7} M	49%**

(ns = not significant; *$p < 0.05$; **$p < 0.01$)

Our experimental data show that, even in low concentrations, these agents directly and differentially modulate the replicative capacity of human plaque SMCs. Additional transmission electron microscopic analysis revealed extensive cell shrinkage, membrane blebbing and formation of apoptotic bodies in SMCs treated with effective, inhibitory doses of nicardipine or nicorandil, signalling typical features of apoptosis.

In conclusion, our experimental results show the inhibitory effects of ion channel modulators on SMC proliferation *in vitro* to be attributable mainly to apoptosis. The described model may be helpful in designing and monitoring candidates for focal drug delivery, before more complex, animal models are used.

1043-101 Amiodarone Inhibits DNA and Protein Synthesis in Human Cardiac Fibroblasts

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Little is known about the effect of Amiodarone, a class III anti-arrhythmic drug on the extracellular matrix in the heart. Amiodarone antagonizes thyroid hormones in various tissues including the heart. We previously showed that the proliferative capacity of cardiac fibroblasts, the matrix-producing cells is regulated by thyroid hormones. In this study we compared the effect of Amiodarone with that of thyroid hormones on DNA and protein synthesis in human cardiac fibroblasts. Cultured human cardiac fibroblasts were prepared from left ventricular tissue of extirpated heart. Cells were treated (24 h) with Amiodarone (5 μg/ml), 3,3',5-triiodothyronine (T_3) (10 nM) or equivalent volume of distilled water (control). The synthesis of DNA and protein was determined by measuring the incorporation of ^{3}H-thymidine and ^{3}H-leucine in cardiac fibroblasts, respectively. The results showed a decrease in the rate of DNA (64%, $p < 0.05$) and protein synthesis (36%, $p < 0.05$) in Amiodarone-treated cells. T_3-treatment, however, led to 40% ($p < 0.05$) increase in DNA synthesis and 30% ($p < 0.05$) decrease in protein synthesis. Amiodarone-treatment of cells in conjunction with T_3 led to a net increase in DNA synthesis and net value of protein synthesis comparable to that in control cells. These data indicate that Amiodarone is an inhibitor of proliferative capacity and metabolic activity of cardiac fibroblasts, thereby a regulator of matrix production. They also point to a functional antagonism between Amiodarone and thyroid hormones. The reversal of amiodarone-induced inhibition of DNA synthesis by thyroid hormones may have clinical relevance in cardiac patients with thyroid gland dysfunction.

1043-102 Antioxidant Dietary Supplementation With Vitamin E Decreased Atherosclerosis and Cellular Proliferation in Lipid-Fed Rabbits Exposed to Passive Smoking

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To evaluate the effects of antioxidants on atherogenesis in a rabbit model of hypercholesterolemia and passive smoking, 32 New Zealand white male rabbits were randomly divided into 4 groups (8 each): Smoke with vitamin E (S + E) or without E (S); No-smoke with vit E (C + E) or without E (C). All four groups were fed 0.3% cholesterol diet for 13 wks. Two vitamin groups were given dietary vit E 1000 IU and beta-carotene 600 mg/kg chow for 21 wks (pretreated 8 wks before receiving lipid diet). Two groups were exposed to passive smoking (4 cigarettes/15 min, 6 hours/day) for 10 wks. Alpha-Tocopherol concentrations were measured in serum, aorta and omentum. Histomorphometry and immunohistochemistry were performed on segments of thoracic aorta using computerized planimetry and specific antibodies.

Group	Alpha-Tocopherol			Aorta		Actin
	Serum (mg/dl)*	Aorta (μg/g)**	Omentum (μg/g)**	Intima (mm^2)*	I/M (%)*	(grade)#
S	2.8 ± 0.6	19 ± 2	18 ± 2	0.14 ± 0.05	4.2 ± 1.6	2.5 ± 0.7
S + E	4.2 ± 1.0	52 ± 9	108 ± 9	0.04 ± 0.03	1.5 ± 1.3	0.3 ± 0.2
C	1.4 ± 0.2	10 ± 1	13 ± 3	0.32 ± 0.19	8.9 ± 5.4	1.9 ± 0.7
C + E	3.6 ± 0.9	54 ± 13	121 ± 11	0.03 ± 0.12	1.2 ± 0.5	2.1 ± 0.7

Values are Mean ± SEM, *$p < 0.06$, *$p < 0.05$, **$p < 0.001$ for E; #$p = 0.05$ for E × S interaction)

Antioxidant dietary supplementation with vitamin E significantly increased alpha-tocopherol concentrations in omentum, aorta and serum; decreased intima lesions (fatty streaks), intima/media (I/M) ratio, and attenuated migration of smooth muscle cells (alpha-actin) which was enhanced by passive smoking.

Conclusion: Antioxidant dietary supplementation with vitamin E decreases atherosclerosis and cellular proliferation in the aorta of hypercholesterolemic rabbits exposed to passive smoking.

1043-103 Effect of Non-Selective Protein Tyrosine Kinase Blocker on Porcine Aortic, Human Arterial, and Atheroma-Derived SMC's Proliferation

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Accelerated proliferative response of smooth muscle cells (SMC's) to vessel wall injury is the principal cause of premature coronary occlusion in patients undergoing heart transplantation, coronary artery bypass grafting, and PTCA. Protein tyrosine kinases (PTK) activity is involved in multiple steps of signal transduction of SMC's growth factors. It is essential for normal cell proliferation, and greatly amplified in proliferative disorders. Blocking the activity of tyrosine kinases may provide a unique and useful strategy for the treatment of syndromes involving accelerated proliferation of vascular SMC's. We examined the inhibitory effect of AG-17, a potent non-specific PTK blocker, on porcine aortic SMC's, human internal mammary artery (IMA) SMC's and human SMC's derived from carotid artery atheromas. 1 uM, 10 uM or 100 uM AG-17 dissolved in 0.1% DMSO was added to the cultures 1, 3 and 5 days after seeding. On day 7 cultures were washed and cells were allowed to recover. Control cultures were treated with 0.1% DMSO. Cells were counted on days 7 and 15. The degree of inhibition of SMC's proliferation is shown in the table. As shown, AG-17 is a very effective inhibitor of SMC's growth. Porcine SMC's were more sensitive to the inhibitory effect of AG-17. 10 uM caused 92% inhibition and 100 uM was toxic to the cells. The inhibition of porcine SMC's proliferation was not reversible, and the cells did not resumed proliferation after day 7. 100 uM AG-17 caused 92% and 70% inhibition of human IMA and human atheroma SMC's respectively and had no toxic effect on the cells. This inhibition of human SMC's was reversible.

% Inhibition of SMC's Proliferation:

SMC's	1 μM	10 μM	100 μM
Porcine	45%	92%	100%
IMA	3%	60%	92%
Atheroma	5%	34%	70%

Conclusions: AG-17, a non-specific PTK blocker is a potent inhibitor of porcine and human SMC's proliferation. In the higher doses tested it had toxic effects on porcine SMC's but not on the human cells.

1043-104 Felodipine Attenuates Monocyte-Endothelial Interaction and Inhibits Intimal Lesion Formation in the Hypercholesterolemic Rabbit

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Calcium entry antagonists have been shown to inhibit the development of new lesions in hypercholesterolemic animals and humans. New lesion formation requires monocyte adhesion to the endothelium. The purpose of this study was to determine if the calcium entry antagonist felodipine inhibited intimal lesion formation by inhibiting monocyte and/or endothelial determinants of adhesion. Male NZW rabbits (n = 28) received the following treatment regimen for 10 weeks: Normal chow (NP, n = 3); normal chow with felodipine infusion (NF, n = 6); 0.5% cholesterol chow (CP, n = 12); or 0.5% cholesterol chow and felodipine infusion (CF, n = 7). After 10 weeks blood was collected for biochemical measurements and mononuclear cell binding assays, and thoracic aortae were harvested for vascular reactivity studies, mononuclear cell binding assays and histomorphometry. In the NF animals, felodipine at this dose did not significantly affect blood pressure, serum cholesterol levels, binding studies, vascular reactivity, or structure. Plasma cholesterol levels were significantly elevated in groups receiving the 0.5% cholesterol diet (N, 29 ± 3; CP, 1221 ± 73; and CF, 979 ± 108 mg/dl respectively), and the adhesiveness of mononuclear cells markedly augmented (by 250%). After thoracic aortae (from CP, n = 5) were incubated with felodipine (10^{-7}) for 1 hour, mononuclear cell adhesion was markedly attenuated (74 ± 9% vs 100%, $p < 0.05$). Impaired endothelium-dependent relaxations (to acetylcholine) in the hypercholesterolemic group were restored by felodipine treatment (40 ± 7% vs 58 ± 4% vs 67 ± 5%; CP vs CF vs NF respectively). This effect was associated with a 2.2-fold reduction in lesion surface area of the thoracic aorta (8.2 ± 6.3% vs 18.2 ± 9.5%; CF vs CP; p = 0.055). Moreover, the intimal-medial ratio reflecting lesion thickness was substantially reduced by felodipine treatment (0.05 ± 0.02 vs 0.17 ± 0.06; CF vs CP; p = 0.025). In conclusion, low-dose felodipine attenuates endothelial determinants of adhesion and inhibits the formation of lesions in hypercholesterolemic animals. Felodipine has direct inhibitory effects upon endothelial adhesiveness; in addition, it enhances vascular nitric oxide activity which may also reduce endothelial adhesiveness.

1043-105 A Synthetic Chlorin Derivative, Tin Ethyl Etiopurpurin, Inhibits Smooth Muscle Cell Proliferation Without Light Activation

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Smooth muscle cell (SMC) proliferation is a major component of restenosis. Hematoporphyrin derivative and other photosensitive compounds (PC's) inhibit proliferation by causing cell necrosis upon light activation (photodynamic therapy, (PDT)). Some PC's, such as benzoporphyrin derivative (BPD), have been suggested to have non-cytotoxic antiproliferative effects without PDT, although the results of several different studies utilizing BPD remain controversial. We investigated the inhibitory effect of a novel photosensitive, synthetic chlorin derivative, tin ethyl etiopurpurin ($SnET_2$), on SMC proliferation without PDT in an *in vivo* model of vascular injury. The iliac arteries of New Zealand White rabbits randomized into 4 groups were ballooned-injured 2 hours post intravenous infusion of placebo (control, (C)) or $SnET_2$ (1, 2, 4 mg/kg). The rabbits were injected 46 hours later with bromodeoxyuridine (BrdU, 30 mg/kg) and then euthanized after 2 hours. SMC proliferation was assessed by quantitation of immunohistochemically detected BrdU incorporation in 5 separate iliac sections obtained from the site of injury. There was a dose-dependent and significant inhibition of SMC proliferation at all concentrations of $SnET_2$ versus C ($p \leq 0.05$). The high dose of $SnET_2$ (4 mg/kg) inhibited SMC proliferation by ≥ 90% of C ($p = 0.02$). The apparent IC_{50} value for half-maximal inhibition occurred over a wide range at 1–2 mg/kg $SnET_2$. Light microscopic examination revealed the absence of any cellular necrosis or nuclear pyknosis in the C or $SnET_2$ treated groups. These results suggest that $SnET_2$ may provide a novel and potent therapy in the treatment of restenosis through inhibition of SMC proliferation without PDT or cytotoxicity.

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Wednesday, March 27, 1996, 3:00 p.m.–5:00 p.m.
Orange County Convention Center, Hall E
Presentation Hour: 3:00 p.m.–4:00 p.m.

1044-6 Hypoxia Enhances Inflammatory Regulation of E-Selectin Through a cAMP-Dependent Pathway

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In many vascular diseases, tissue hypoxia occurs in conjunction with other inflammatory processes. Given the involvement of leukocytes in tissue damage during reperfusion injury, we hypothesized that hypoxia may differentially regulate expression of an important leukocyte adhesion molecule, E-selectin (ELAM-1). Bovine aortic endothelial monolayers were exposed to hypoxia (pO_2 3 torr) in the presence or absence of tumor-necrosis factor-α (TNF-α) or lipopolysaccharide (LPS), known regulators of E-selectin. Endothelial cell surface E-selectin was quantitated by whole cell ELISA and by immunoprecipitation using polyclonal anti-E-selectin sera. Endothelial mRNA levels were assessed using ribonuclease protection assays. TNF-α or LPS induced a time- and dose-dependent induction of specific E-selectin surface expression under normoxic conditions. Hypoxia per se, did not induce endothelial E-selectin expression, however, the combination of TNF-α or LPS and hypoxia resulted in enhanced induction of endothelial E-selectin expression (85 ± 10 and 105 ± 8% over LPS and TNF-α alone, respectively, both $p > 0.001$). Densitometry of immunoprecipitations from hypoxia/LPS revealed a 270% increase over normoxia/LPS. mRNA levels of hypoxic endothelia were increased 190% over normoxia with addition of TNF-α, but were not increased by hypoxia alone. Finally, we and others have shown an hypoxia-elicited decrease in endothelial cAMP (>50%). Addition of forskolin (cAMP agonist, 10 μM) and isobutyl-methyl-xanthine (phosphodiesterase inhibitor, 5 mM) during hypoxia resulted in normalization of cAMP and a loss of enhanced E-selection surface expression with TNF-α and hypoxia. We conclude that hypoxic stress enhances LPS/TNF-α induction of E-selectin and is, at least in part, cAMP-dependant. From such data, we speculate that hypoxic stress may represent a pathophysiologically-relevant stimuli during conditions of inflammation.

1044-107 Selective Activation of Endothelin Receptor (ETR) Promoters During Differentiation: Analysis of Cis-Acting Elements

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The interaction of ETs with their receptors plays a critical role in the development of cardiac and vagal neural crest-derived structures including the interventricular septum, outflow tract and great vessels (ET-1/ET_A), and ganglionic neurons (ET-3/ET_B). We have shown the selective induction of ET_A or ET_B during differentiation of the pluripotential embryonal cell line P19 to a cardiomyocyte or a neural lineage, respectively. In order to define tissue-specific elements, P19 cells were transfected with ET_A or ET_B promoter-luciferase constructs. The region −848 to +92 of ET_A induced luciferase expression in the cells differentiated to a myocyte lineage but not in the undifferentiated cells. Deletion analysis identified two strong positive regulatory elements (−116 to −38 and +90 to +200), both of which were able to markedly enhance the activity of a heterologous promoter in cells expressing ET_A. Selective induction of the ET_B-luciferase construct with differentiation to a neural lineage was seen with the fragment −1100 to +250 of the ET_B gene, and deletion of potent negative regulatory elements (−1100 to −200) produced a 23-fold increase in promoter activity. Conclusions: 1) There is selective differentiation-dependent activation of cis-acting elements in the ETR promoters in a model system resembling morphologically and biochemically key events of early embryogenesis; 2) Positive differentiation-specific as well as negative regulatory elements are involved.

1044-108 Cell-Specific Interaction of Nuclear Factors With a Cis-acting Negative Regulatory Element in the Rat Cardiac Alpha Myosin Heavy Chain Gene

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The alpha myosin heavy chain (α-MHC) gene encodes an isoform of a major contractile protein of the heart, the myosin heavy chain. Expression of α-MHC